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Pegivirus Infection in Domestic Pigs, Germany

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To the Editor: The family *Flaviviridae* includes many human and animal virus pathogens. Recently, in addition to the genera *Flavivirus*, *Hepacivirus*, and *Pestivirus*, a fourth genus, *Pegivirus*, has been identified (*1*). In addition to human pegiviruses, a range of phylogenetic, highly divergent pegiviral sequences have been identified in various animal species, including primates, bats, rodents, and horses (*2*). We report the detection of a porcine pegivirus (PPgV) in serum samples from pigs.

Initially, we investigated pooled serum samples by using high-throughput sequencing methods and isolated RNA from individual porcine serum samples by using the QIAmp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). We prepared libraries compatible with Illumina (San Diego, CA, USA) sequencing from pooled samples and individual serum samples by using the ScriptSeq version 2 RNA-Seq Library Preparation Kit (Epicenter, Madison, WI, USA) and sequenced them by using a HiSeq 2500 (2 × 150 cycles paired-end; Illumina) for pooled samples and MiSeq (2 × 250 cycles paired-end; Illumina) for individual samples (*3*).

We conducted quantitative reverse transcription PCR (RT-PCR) by using a Quantitect-SYBR Green Assay (QIAGEN) and primers PPgV_fwd: 5′-CTGTCTATGCTGGTCAC-GGA-3′ and PPgV_rev: 5′-GCCATAGAACGGGAAGTC-GC-3′. By using high-throughput sequencing of the pooled serum sample library (23,167,090 reads), we identified 1 contig (4,582 bp) that had distant nucleotide sequence similarity to bat pegivirus (69% and 4% sequence coverage) and 2 contigs (2,683 bp and 665 bp) that had 73% sequence coverage, thereby covering 8% and 37% of the identified sequence. RT-PCR with primers designed on basis of recovered sequences identified the sample containing pegivirus sequences. Subsequent MiSeq analysis (7,085,595 reads) of an RNA library prepared from a sample from 1 animal identified 1 contig (9,145 nt) with sequence similarity to pegivirus sequences.

We performed 3′ end completion of the viral genome by rapid amplification of cDNA ends and identified the entire open reading frame of PPgV_903 encoding 2,972 aa (GenBank accession no. KU351669). Analysis of the pegivirus 5′ untranslated region identified a highly structured internal ribosome entry site motif (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/7/16-0024- Techapp1.pdf), which was similar in structure to previously described 5′ untranslated region structures of other pegiviruses (*4,5*).

Pegiviruses do not encode a protein homologous to the capsid protein of other viruses of the family *Flaviviridae*, another common feature of pegiviruses (*6*). The presence of cleavage sites for cellular signal peptidases and viral proteases indicates that, similar to polyproteins of other pegiviruses and members of the genus *Hepacivirus*, the pegivirus polyprotein NH_2-E1- E2-Px-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH (E [envelope], NS [nonstructural],
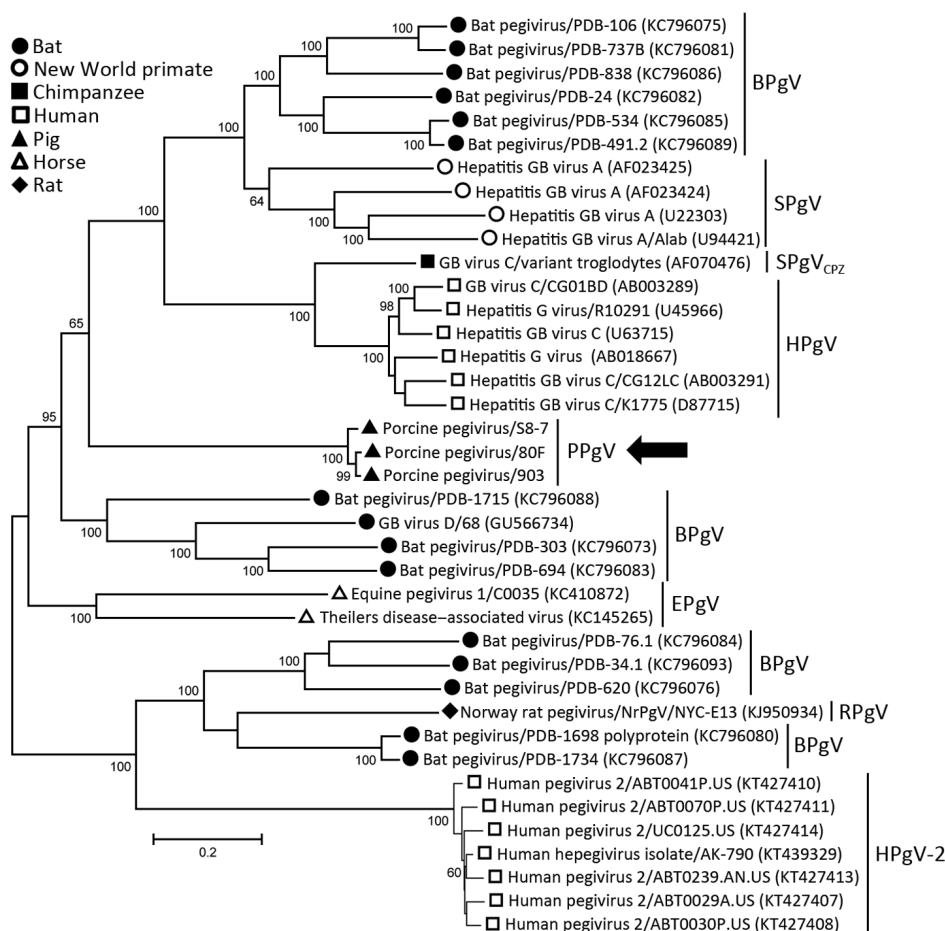
Figure. Phylogenetic analysis of human and animal pegiviruses. We constructed a maximum-likelihood tree on the basis of the complete coding region and used the general time reversible model for modeling of substitutions. Bootstrap analysis was performed with 200 replicates. Numbers along branches are percentage bootstrap values. GenBank accession numbers are in parentheses. Arrow indicates viruses isolated in this study. Scale bar indicates nucleotide substitutions per site. BPgV, bat pegivirus, SPgV, simian pegivirus; SPgV$_{CPZ}$, simian pegivirus (chimpanzee); HPgV, human pegivirus; PPgV, porcine pegivirus; EPgV, equine pegivirus; RPgV, rodent pegivirus. GB viruses have recently been reclassified as pegiviruses.

and Px [protein X]) is cleaved co-translationally and posttranslationally.

We tested 3 additional animals from the same breeding cohort for virus RNA at irregular intervals for 22 months. One animal was positive for pegivirus RNA for 7 months, and the other 2 animals had pegivirus RNA in serum for 16 and 22 months. None of these animals showed obvious clinical signs attributable to virus infection. Follow-up investigation of 455 serum samples from 37 swine holdings from Germany identified 10 (2.2%) samples from 6 pig holdings that contained pegivirus RNA. We obtained 2 additional near full-length genomic sequences (PPgV_80F and PPgV_S8-7) from 2 animals in different herds by high-throughput sequencing, RT-PCR, and Sanger sequencing (GenBank accession nos. KU351670 and KU351671).

Phylogenetic analyses of complete coding regions showed the close relationship of the 3 pegivirus sequences from Germany. These 3 sequences formed a separate clade within the genus *Pegivirus* (Figure). Pairwise comparison between PPgV_903 and the other 2 pegivirus sequences showed strong nucleotide identities (96.0%–98.4%). A distance scan over the entire polyprotein showed genetic distance to other pegiviruses and demonstrated that NS3 and NS5B contain the most conserved regions among pegivirus polyproteins (online Technical Appendix).

In horses, 2 distinct pegiviruses that had different potentials to cause clinical disease in infected animals have been described (*4,7*). No obvious clinical effects were observed in pegivirus-infected animals during our study. However, potential consequences of viral infection for animal health and food production need to be explored more closely under field and experimental conditions. Pegiviruses can interact with the immune system of the host. Coinfection with human pegivirus and HIV can have beneficial effects, which result in decreased retroviral loads and delayed disease progression (*8*).

It will be useful to investigate whether co-infections with pegiviruses can influence clinical manifestations of infectious diseases of swine, including multifactorial diseases such as postweaning multisystemic wasting syndrome, in which unknown immune modulating virus infections have been suggested to influence the degree of clinical illness (*9*). RNA viruses have considerable potential to adapt to new environmental conditions and to overcome host restrictions (*10*). Until now, the host tropism of PPgV has not been investigated in detail. Therefore,

additional studies will be required to elucidate whether the spectrum of potential hosts might include other farm or companion animals, and whether the virus might be able to infect humans.

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New Chimeric Porcine Coronavirus in Swine Feces, Germany, 2012

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To the Editor: Porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV) can cause severe enteritis in pigs accompanied by diarrhea, vomiting, and dehydration. Clinical signs are most prominent in young suckling pigs, in which high mortality rates are common. As seen in recent porcine epidemic diarrhea outbreaks in the United States and Asia, the effect on the pig industry can be tremendous.

Recently, Boniotti et al. (*1*) reported detection and genetic characterization of swine enteric coronaviruses (CoVs) circulating in Italy during 2007–2014. Characterization was based on sequencing and phylogenetic analyses of spike genes of TGEV and PEDV isolates. This study also reported a new recombinant CoV strain with a TGEV backbone and a PEDV spike gene (SeCoV/Italy/213306/2009; KR061459), which was identified as a swine enteric CoV (SeCoV). This chimeric virus presumably resulted from a recombination event.

Accompanying a study of recent porcine epidemic diarrhea cases in Germany caused by a new PEDV Indel strain (*2*), we retrospectively analyzed fecal samples from pigs that showed typical clinical symptoms of a PEDV infection. The sample set included fecal material collected from a farm in southern Germany on which an episode of diarrhea among pigs occurred in 2012. This material was shown by electron microscopy to contain CoV-like particles (Figure), but showed negative results by reverse transcription PCRs specific for the PEDV nucleocapsid gene.

Subsequent metagenomic analyses resulted in the full-genome sequence of a swine enteric CoV (SeCoV/GER/L00930/2012). We found a sequence showing high similarity (99.5% identity) with the TGEV/PEDV recombinant reported by Boniotti et al. (*1*). Network analysis of complete genome sequences of similar CoVs underline the chimeric nature of the genome between TGEV and PEDV genome sequences (online Technical Appendix Figure,

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Technical Appendix

We used Trimmomatic (*1*) to remove adaptor sequences and trim low-quality bases (phred quality score <15) from the 3′-end of the reads. The reads were then aligned to the assembled consensus sequence of porcine pegivirus PPgV_903 with the Burrows Wheeler Aligner (bwa mem) (*2*). Samtools (*3*) was used for format conversion, sorting, and removal of putative PCR duplicates. Variants were called with FreeBayes (*4*). The minimal fraction of observations required to call an alternate allele was reduced to 5%, whereas the minimum absolute number of required observations was increased to 5. To increase stringency, FreeBayes' standard filters were used and results were filtered to exclude variants with a quality score <21.

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Table. Variant calling for genomes of pegiviruses*

Position	PPgV_903 reference†	Variant sequence‡	Variant frequency PPgV_903§	Variant frequency PPgV_80F§
321	C	T	0	1
330	T	A	0.36	0
559	A	G	0	0.97
704	T	C	0	1
755	C	T	0	1
767	C	T	0	1
795	A	T	0	1
858	T	C	0	1
867	G	T	0.62	0
869	C	T	0	1
914	A	G	0.71	0
980–983	CAGC	TAGT	0	1
1098	A	G	0	1

Position	PPgV_903 reference†	Variant sequence‡	Variant frequency PPgV_903§	Variant frequency PPgV_80F§
1170	C	T	0.03	1
1181	T	C	0	0.67
1196–1199	TCTG	CCTA	0	0.95
1226	C	T	0.06	1
1265	T	C	0	1
1271	C	T	0	1
1455	T	C	0	1
1465	T	A	0.17	0
1469	T	C	0	1
1511	C	T	0	1
1522	T	C	0.44	0
1539	C	T	0	1
1574	C	T	0	1
1586	T	C	0	1
1646	T	C	0.16	0
1718	C	T	0	1
1727	T	C	0	1
1832	C	T	0	1
1895	T	C	0	1
1901	C	T	0	1
1907	T	C	0.02	1
2057	A	G	0	1
2066	G	A	0	0.98
2096	C	T	0	1
2102	C	T	0	1
2195	C	T	0.07	1
2231	T	C	0	1
2300	T	C	0	1
2375	C	T	0	1
2545	A	G	0	1
2634	C	T	0	0.87
2672	C	T	0	1
2732	C	T	0	1
2741	T	C	0.03	0.96
2837	T	C	0	1
2891	T	C	0	1
3173	T	C	0.12	0.02
3182	C	T	0	1
3260–3262	CAA	TAG	0	1
3378	T	C	0	1
3467	C	T	0.03	1
3575	T	C	0	0.84
3587	C	T	0.38	0
3614	G	A	0	1
3764	G	A	0	1
3911	T	C	0	1
3917	A	T	0.36	0
4001	T	C	0	1
4013	T	C	0	1
4097	C	T	0.19	0
4160	C	T	0	1
4181	A	G	0.11	1
4196	T	C	0	1
4211	G	A	0	0.9
4346	C	T	0	0.48
4424	C	T	0	1
4478	C	T	0	1
4541	A	G	0	0.99
4700	A	G	0	0.38
4784	G	A	0	1
4817	C	T	0.26	0
4835	T	C	0	0.98
4940	A	G	0	1
4985	T	C	0	1
5114	C	T	0.07	1
5192	T	C	0	1
5441	T	C	0	0.98
5474	A	G	0	1
5492	T	C	0.44	1
5732	T	C	0	1
5936	C	T	0	1
5999	G	A	0	1
6074–6077	CGTT	TGTC	0	1
6128	A	G	0	1
6191	C	T	0	1

Position	PPgV_903 reference†	Variant sequence‡	Variant frequency PPgV_903§	Variant frequency PPgV_80F§
6220	G	A	0	0.82
6297	C	T	0	1
6323	C	T	0	1
6338	T	C	0.05	1
6362	T	C	0.33	1
6389	T	C	0	1
6449	C	G	0	1
6482	T	C	0	1
6509	C	T	0	1
6542	A	G	0	1
6554	T	C	0	1
6632	T	C	0.38	0
6722	T	C	0	1
6744	T	C	0	1
6761–6764	TTTG	CTTA	0	1
6770	G	T	0	1
6803–6807	CGTGA	TGTGG	0	1
6824	C	T	0.15	0
6878	T	C	0	1
6892	T	C	0.53	1
6926	A	G	0.01	0.98
6965	T	C	0.01	0.89
7088	A	G	0	1
7343	G	A	0	0.9
7349	T	C	0	1
7364	T	C	0	1
7460–7466	AGCGACG	GGCAACA	0	1
7493	C	T	0	1
7517	T	C	0	0.92
7601	T	C	0.05	1
7662	C	T	0.04	1
7694	T	C	0	1
7745	T	C	0	1
7751	A	G	0.25	0
7763	A	C	0	1
7931	T	C	0.05	0.87
7967	A	G	0	0.95
7997	C	T	0.13	0
8036	G	A	0.01	1
8129	A	G	0	1
8207	T	C	0	1
8309	C	T	0.24	0.04
8330	T	C	0	0.98
8351	C	T	0.16	0
8384	G	A	0	1
8435	A	G	0	1
8522	C	T	0	1
8537	G	A	0	1
8544	G	A	0	1
8549	A	G	0	1
8566	G	A	0	1
8573	T	C	0.06	1
8591	C	T	0	1
8600	C	T	0.03	1
8630	A	C	0	1
8639	A	G	0	1
8666	C	T	0.28	0.02
8708	C	T	0.03	1
8738	T	C	0.12	0.91
8747	T	C	0	1
8789	T	C	0	1
8828	T	C	0	0.67
8930	T	A	0	1
9032	C	T	0.02	1
9050	T	C	0	1
9077	C	T	0.06	1

*PPgV, porcine pegivirus.
†Reference nucleotide sequence as assembled from PPgV_903 sequencing reads. The sequence served as a common reference for the analysis of sequence variants in PPgV_80F- and PPgV_903-infected animals.
‡Variant nucleotide sequence as observed in PPgV_80F- or PPgV_903-infected animals.
§Frequency of variant nucleotide sequences in PPgV_80F or PPgV_903, respectively (0, 0%; 1, 100%).
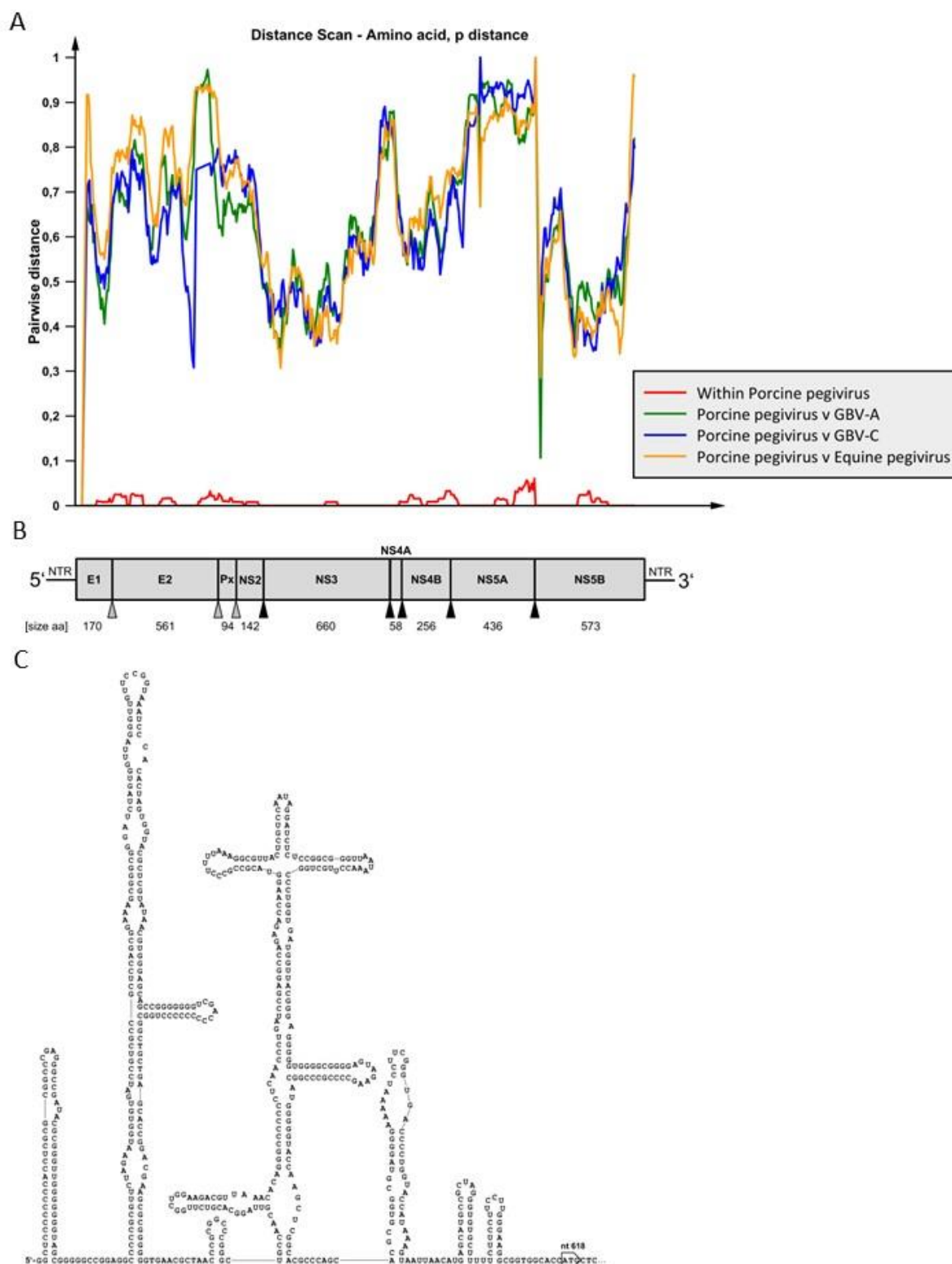
Technical Appendix Figure. A) Pairwise amino acid distances plotted over the complete polyprotein of pegiviruses. A schematic drawing of the pegivirus polyprotein is depicted below indicating corresponding genomic regions. GBV, hepatitis G virus. B) Schematic drawing of the pegivirus polyprotein. Putative cleavage sites specific for cellular signal peptidases are indicated in gray, and cleavage sites typical for viral proteases are indicated in black. The length of each putative mature viral protein is given below. E1, E2, envelope glycoproteins; P, p protein; NS, nonstructural proteins.

C) Schematic drawing of the pegivirus internal ribosome entry site structure within the 5′-nontranslated region (NTR). RNA folding was performed by using mfold (http://unafold.rna.albany.edu/?q=mfold/rna-folding-form) (default settings). The box indicates the start codon of the polyprotein.